Supplementary Text

The importance of drift and selection

To determine whether the change in major allele frequency and diversity spectra can be explained by drift alone or whether selection must be invoked, we conducted two analyses.

First, we compared the distribution of diversity measures in the treatment samples (glycerol and glycerol + freezing) to that expected under a pure drift model. To do so, we constructed a null distribution that reflects how subsampling alone affects the diversity measure. If the glycerol and glycerol + freezing cultures fall into this null distribution, then we can say that drift is sufficient to explain the observed diversity metric spectra. We constructed the null distribution of the diversity measure by resampling the allele frequency 10 times for 100 randomly selected genes from the initial culture. To compare the distributions of the resampled initial cultures and the glycerol and glycerol + freezing samples, we plotted the cumulative distribution for the diversity metric (Figure 3A) and the fraction of a sites in the genome that have a given diversity metric for each sample (Figure 3B). Because the expected distribution of diversity values under drift alone in the resampled initial cultures differs from the treatments, drift alone cannot explain the observed spectra of variation in diversity metrics.

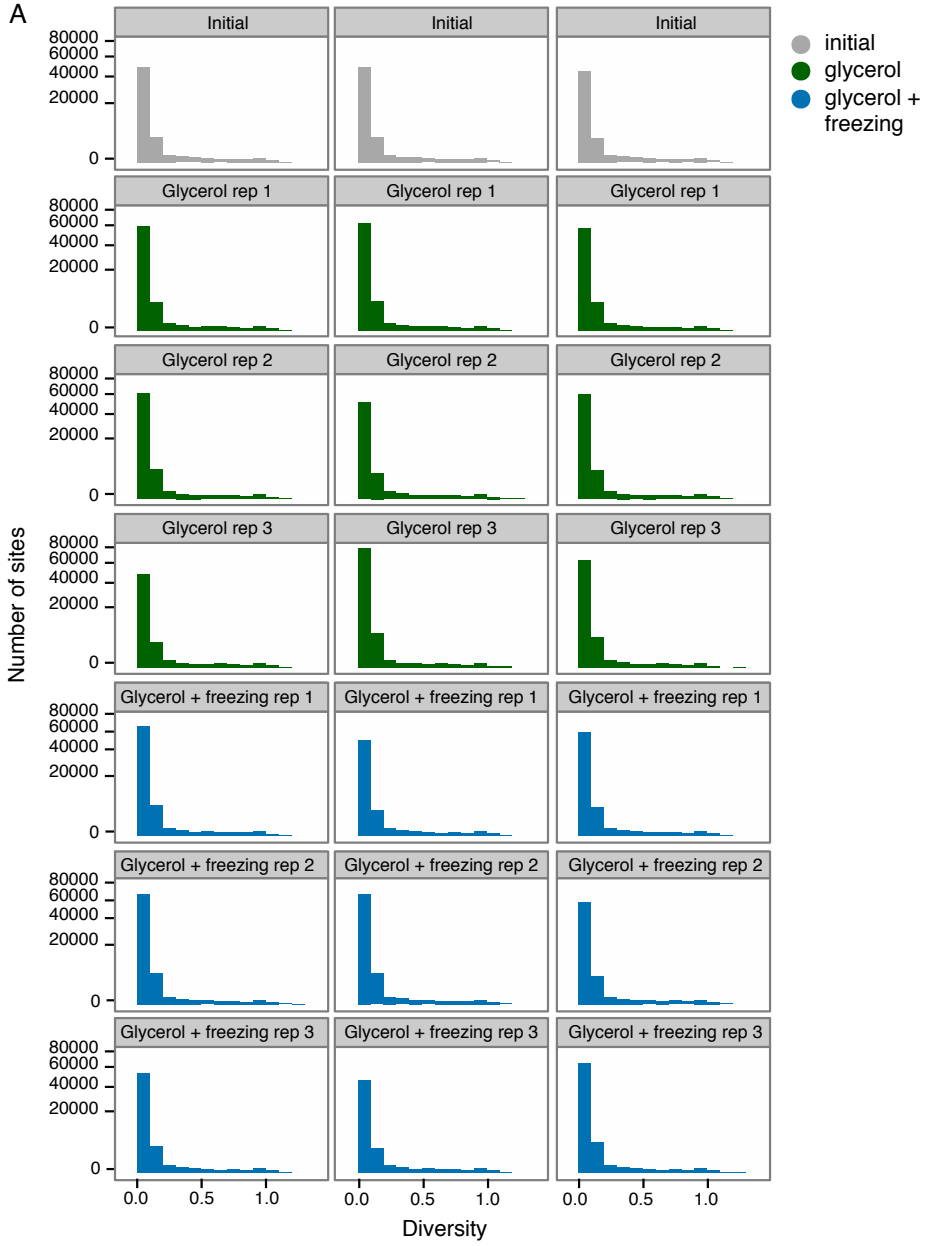
Next, we computed the ratio of the effective population size to census size (N_e / N) (Luikart et al. 2010) for all of the subsamples from the three treatments. When N_e / N is very small, drift can have a strong effect even though the population may be large (Nunney 1995). To determine the expected distribution of the N_e / N ratio under drift alone, we computed the ratio for resamples from the initial treatment. We selected 100 random genes and obtained the allele frequency for all sites in these genes. For each sequenced initial subsample, we generated 50 resamples of the allele frequency with replacement. For all experimental and resampled samples, we computed N_e using the Lynch and Conery method for haploids (Lynch and Conery 2003). In short, we obtained N_e using $N_e = \frac{1.5H}{\mu(3-4H)}$, where $\mu = 8.9 \times 10^{-11}$ is conservatively the wildtype mutation rate (Wielgoss et al. 2011), H is the expected

heterozygosity given by the average fraction of synonymous changes between two randomly selected sequences and was obtained under the assumption of random codon usage by $H = {}^{\pi}/_{4}$ (following Lynch and Conery (Lynch and Conery 2003)) and π is the nucleotide diversity, or the average pairwise fractional distance of sequences (Grauer and Li 2000) in a 20 bp sliding window. We obtained the census population size N using the harmonic mean of the cell counts during the course of the experiment, and finally plotted the ratio of the effective population size to census size (Figure S2). If drift alone was sufficient to generate the N_e / N ratio observed in the treatment subsamples (glycerol, glycerol + freezing), then we would expect similar values for the 50 initial resamples and the treatments. Instead, our 50 computational resamples of the initial treatment differ from the experimental treatments (glycerol and glycerol + freezing). Thus, we can conclude that drift is not sufficient to generate the change in the N_e / N ratio.

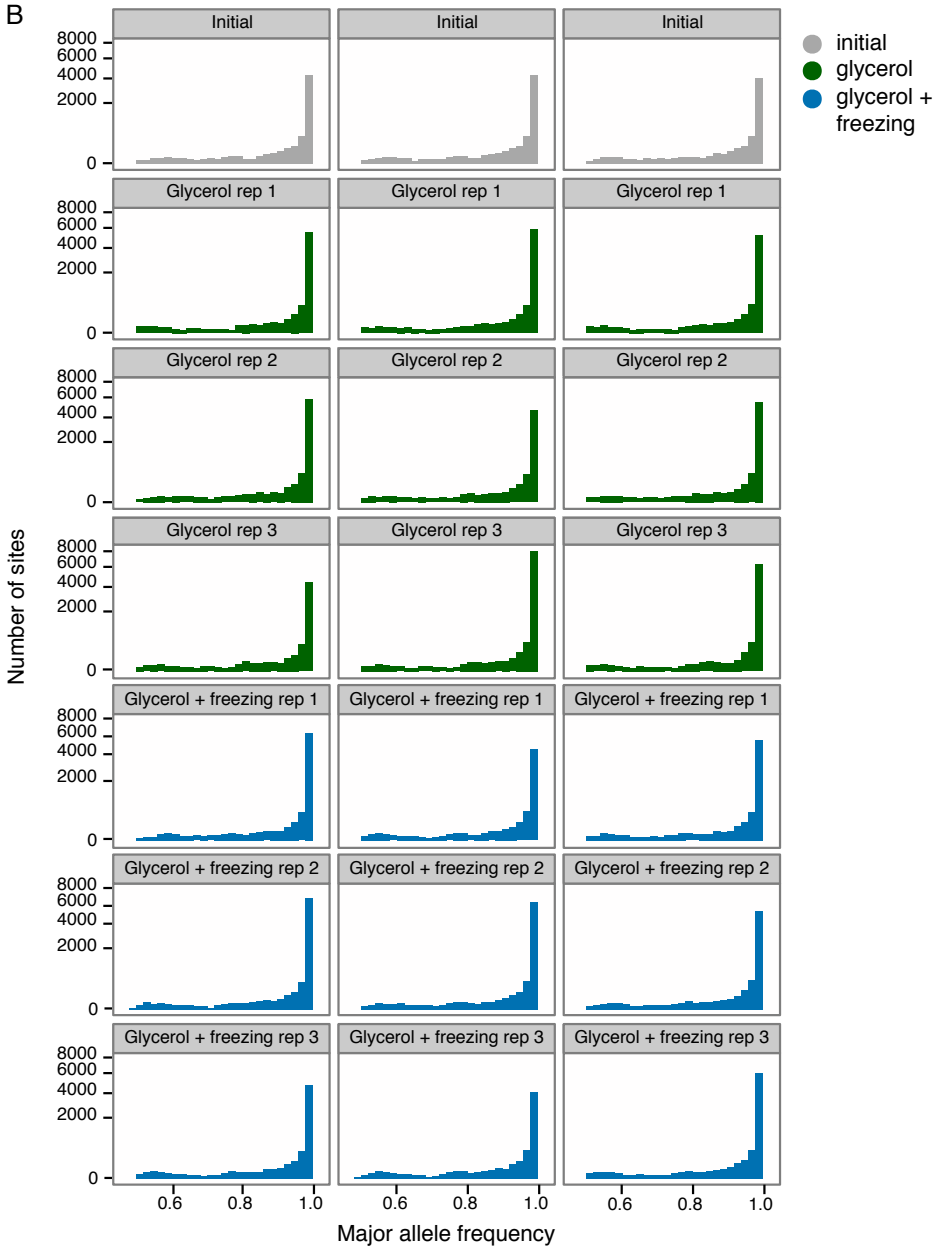
The analyses presented in the preceding paragraphs suggest that selection may be more important than drift in treating *E. coli* samples with glycerol and / or glycerol + freezing.

Supplementary Figures

A



B


Initial
Initial
Initial
Glycerol rep 1
Glycerol rep 1
Glycerol rep 1
Glycerol rep 2
Glycerol rep 2
Glycerol rep 2
Glycerol rep 3
Glycerol rep 3
Glycerol rep 3
Glycerol + freezing rep 1
Glycerol + freezing rep 1
Glycerol + freezing rep 1
Glycerol + freezing rep 2
Glycerol + freezing rep 2
Glycerol + freezing rep 2
Glycerol + freezing rep 3
Glycerol + freezing rep 3
Glycerol + freezing rep 3
8000
6000
4000
2000
0
0.6
0.8
1.0
Number of sites
Major allele frequency
initial
glycerol
glycerol + freezing

1

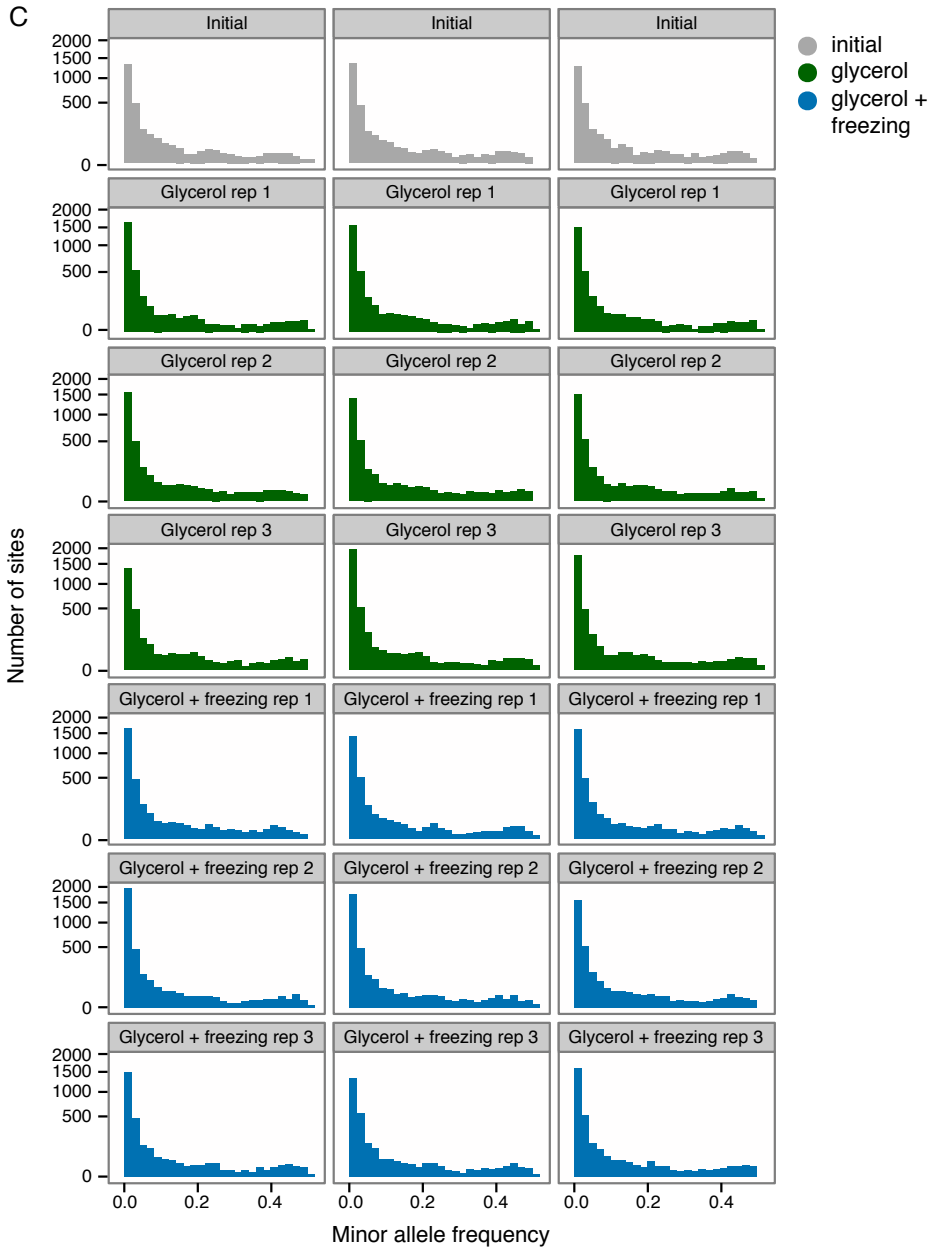


Figure S1. We plotted the (A) diversity, (B) most abundant allele frequency, and (C) minor allele frequency for the initial samples and treatment with glycerol or glycerol and freezing (treatments are labelled in gray). The minor allele frequency was obtained by $\min(p_i)$, where p_i is the frequency of allele *i* at a given site (determined as the fraction of reads with allele *i*). We conservatively considered only sites with diversity metrics greater than 0.0671, major alleles that were observed at most in 247/250 reads, or minor alleles that were observed in at least 3/250 reads. We tested whether the combined distributions from a given culture were different from those from all other cultures, and the results are given in Table 1.

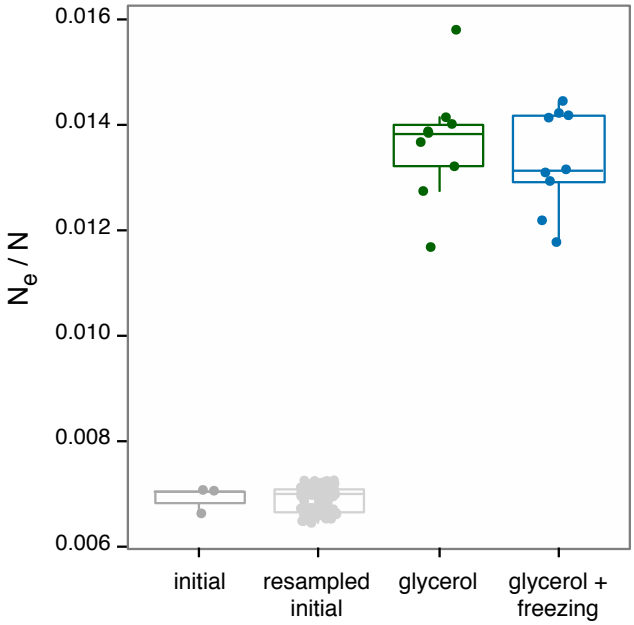


Figure S2. The ratio of the effective population size (N_e) to the census population size (N) for the three experimental treatments (initial, glycerol, and glycerol + freezing) and the expectation of this ratio under pure drift obtained by resampling the allele frequency from initial subsamples ("resampled initial"). Resampling mimics the expected effect of drift on the N_e / N ratio. Each point corresponds to the N_e / N ratio for 100 randomly-selected genes, obtained from experimental data ("initial", "glycerol", and "glycerol + freezing") or from 150 resamples from the initial culture ("resampled initial"). The full methods are described in the Supplementary Text.

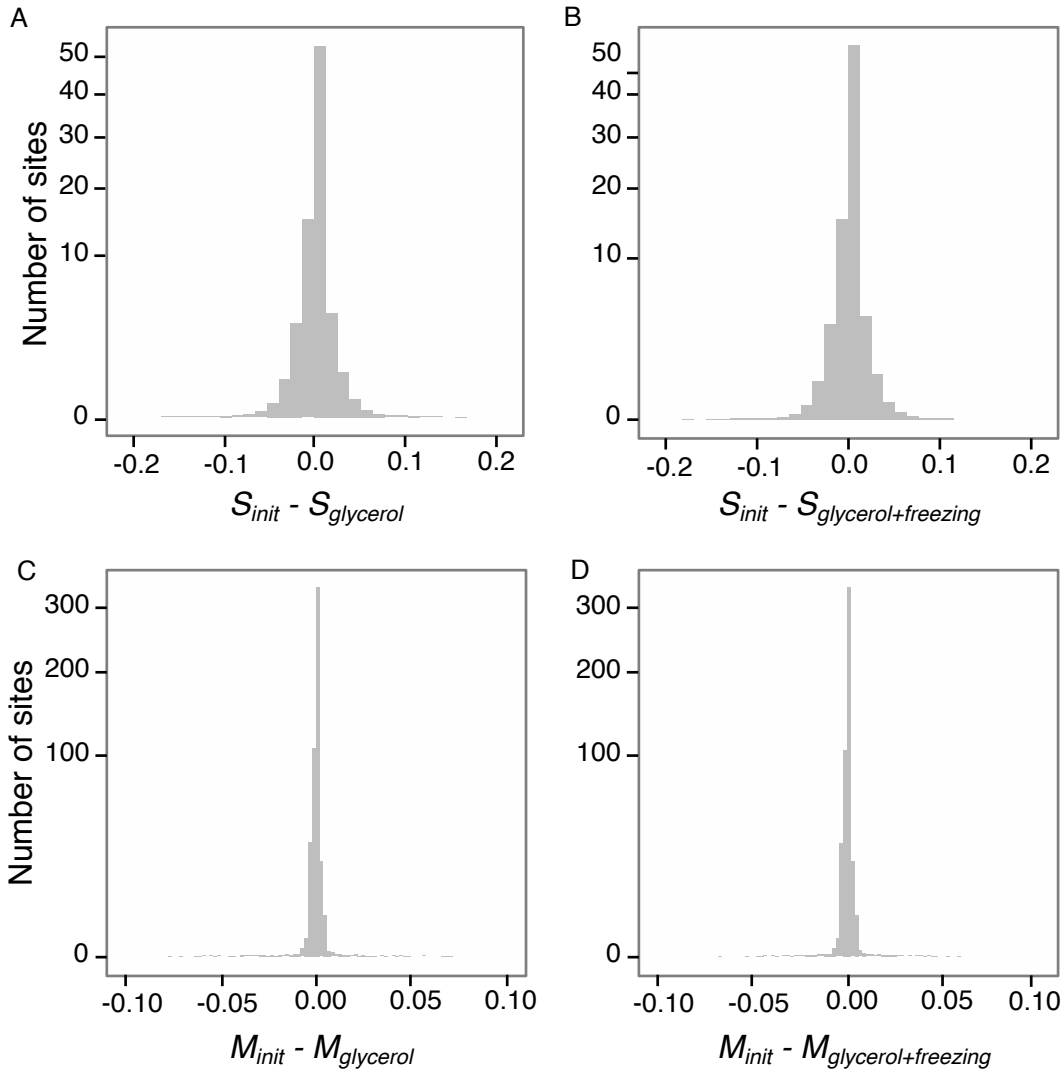
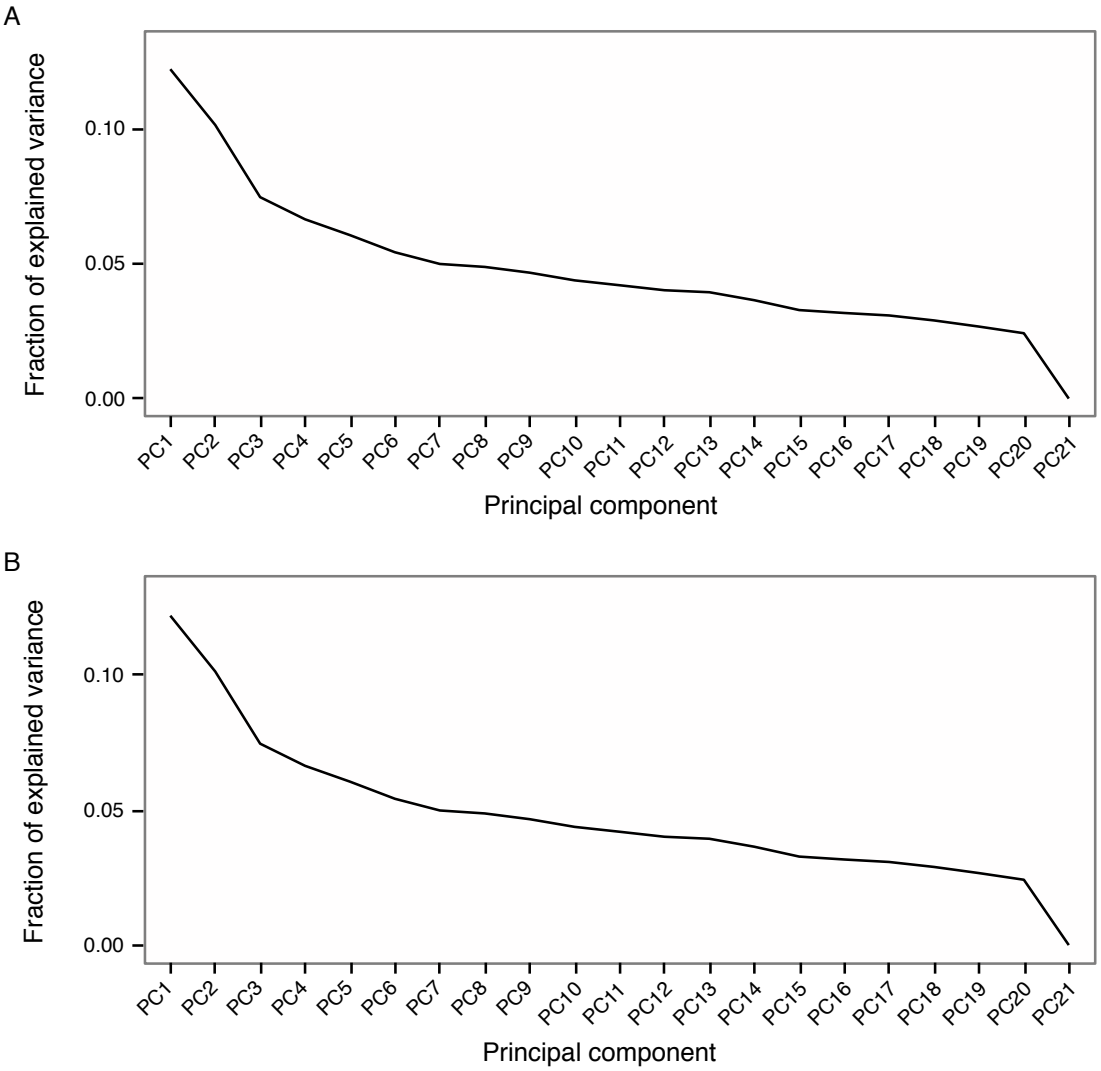


Figure S3. The change in the diversity index, *S*, after treatment with (A) glycerol or (B) glycerol + freezing, and the change in frequency of the most abundant allele, *M*, after treatment with (C) glycerol or (D) glycerol + freezing. (A,B) Positive values mean that the diversity increased in comparison to the initial sample. (C,D) Positive values mean that the most common allele increased in frequency in comparison to the initial sample, while negative values mean that it decreased.



1

2 Figure S4. The fraction of total variance explained by each principal component from the PCA

3 analysis using (A) the diversity measure, and (B) the frequency of the most abundant allele.

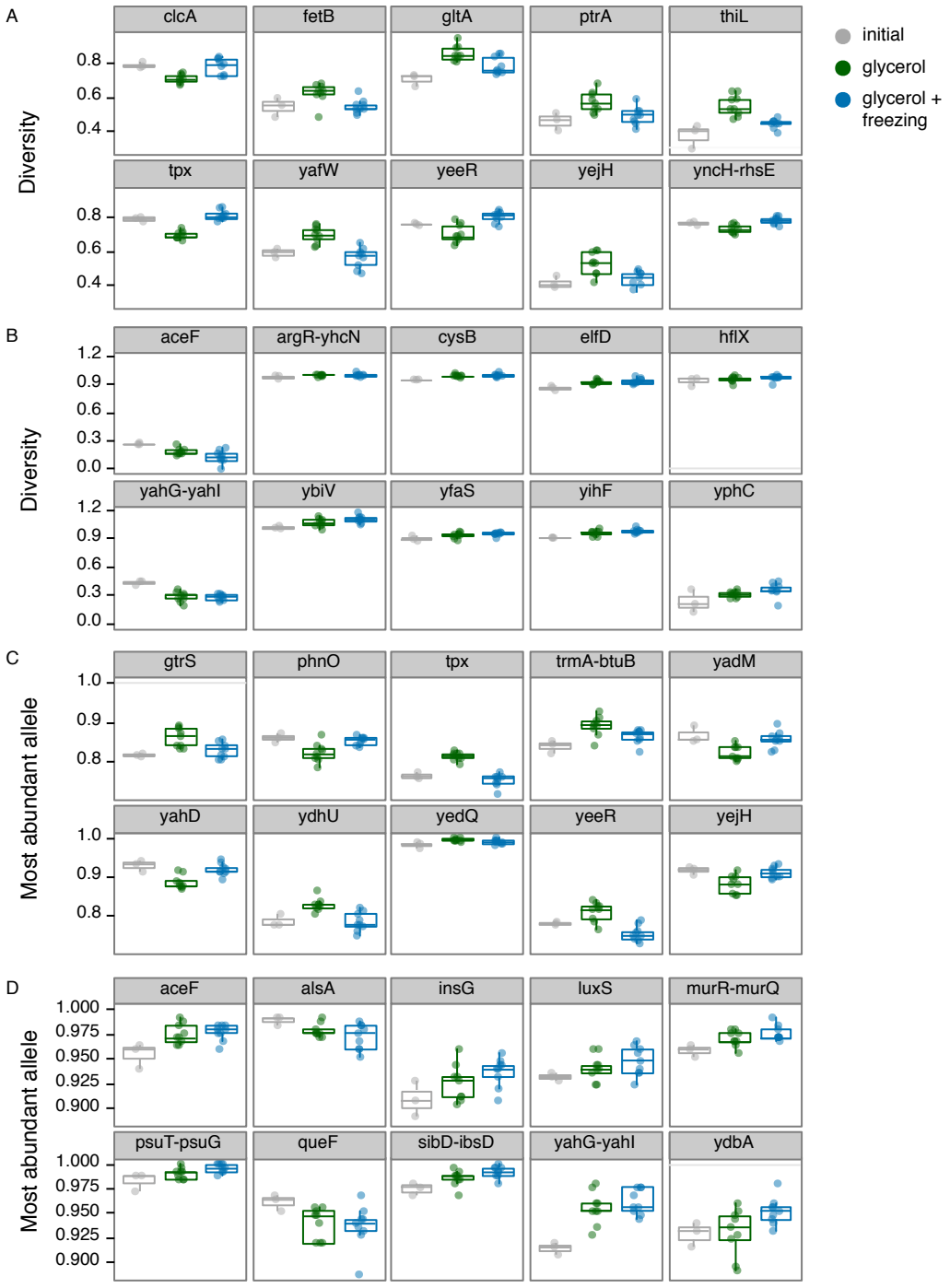


Figure S5. Plots of the diversity measure and the frequency of the most abundant allele for the ten genes that contributed most strongly to the principal components PC1 and PC2. The genes important for (A) PC1 and (B) PC2 for the diversity measure, and the genes important for (C) PC1 and (D) PC2 for the most abundant allele. Gene names (in gray boxes) consisting of two hyphenated genes indicate that the site is in the intergenic region between two genes. Initial samples are gray, glycerol samples are green, and glycerol + freezing samples are blue.

Supplementary Table

Diversity

	C6	C7	C8	F6	F7	F8
II	4.00E-13*	2.43E-06*	6.52E-10*	6.99E-05*	1.10E-14*	1.27E-04*
C6		0.121	1.00	0.841	1.00	0.0397
C7			0.391	1.00	7.93E-04*	0.997
C8				0.151	1.00	0.0278
F6					0.0324	1.00
F7						3.04E-07*

Most abundant allele frequency

	C6	C7	C8	F6	F7	F8
II	2.02E-12*	6.87E-07*	8.54E-12*	2.74E-06*	9.01E-10*	2.78E-03
C6		0.923	1.00	0.0289	0.962	8.55E-05*
C7			0.877	0.906	0.379	0.295
C8				0.104	1.00	0.137
F6					0.254	0.311
F7						1.13E-03*

Minor allele frequency

	C6	C7	C8	F6	F7	F8
II	7.43E-08*	8.03E-08*	1.89E-12*	2.27E-04*	1.32E-14*	1.52E-06*
C6		0.338	1.00	0.0716	0.991	0.00766
C7			2.71E-01	1.00	0.209	0.174
C8				1.35E-04*	1.00	0.0850
F6					0.962	0.899
F7						4.74E-05*

Table 1. P-values highlighting the significant differences in metric distributions between cultures. We combined the three subsamples for each culture and tested all pairwise combinations of cultures for each metric to determine if the values could be drawn from the same distribution using the Kolmogorov-Smirnoff test. We determined significance after using the Bonferonni correction for multiple testing (starred and highlighted in dark gray). In general, the tests showed that the initial culture was not drawn from the same distribution as any glycerol or glycerol + freezing cultures.

Supplementary References

Grauer D, Li W. 2000. Fundamentals of molecular evolution. Sinauer

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conserv Genet 11:355–373.

Lynch M, Conery JS. 2003. The origins of genome complexity. Science 302:1401–1404.

Nunney L. 1995. Measuring the Ratio of Effective Population-Size to Adult Numbers Using Genetic and Ecological Data. Evolution 49:389–392.

Wielgoss S, Barrick JE, Tenaillon O. 2011. Mutation rate inferred from synonymous substitutions in a long-term evolution experiment with Escherichia coli. G3: Genes| Genomes| Genetics 1:183–186.